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A New and Frequent Human T-Cell Leukemia Virus Indeterminate Western Blot Pattern: Epidemiological Determinants and PCR Results in Central African Inhabitants

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Human T-cell leukemia virus (HTLV) indeterminate Western blot (WB) serological patterns are frequently observed in plasma/serum from persons living in intertropical areas. In the framework of ongoing projects on HTLV-1/2 and related viruses in Central Africa, we systematically analyzed plasma from villagers living in South Cameroon by WB. The group included 1,968 individuals (mean age, 44 years; age range, 5 to 90 years; 978 women/990 men), both Bantus (1,165) and Pygmies (803). Plasma samples were tested by WB analysis (MPD HTLV Blot 2.4) and interpreted according to the manufacturer's instructions. Only clear bands were considered in the analysis. Among the 1,968 plasma samples, 38 (1.93%) were HTLV-1, 13 (0.66%) were HTLV-2, and 6 (0.3%) were HTLV WB seropositive. Furthermore, 1,292 (65.65%) samples were WB sero-indeterminate, including 104 (5.28%) with an HTLV-1 Gag-indeterminate pattern (HGIP) and 68 (3.45%) with a peculiar yet unreported pattern exhibiting mostly a strong shifted GD21 and a p28. The other 619 (31.45%) samples were either WB negative or exhibited other patterns, mostly with unique p19 or p24 bands. DNA, extracted from peripheral blood buffy coat, was subjected to PCR using several primer pairs known to detect HTLV-1/2/3/4. Most DNAs from HTLV-1- and HTLV-seropositive individuals were PCR positive. In contrast, all the others, from persons with HTLV-2, HGIP, new WB, and other indeterminate patterns, were PCR negative. Epidemiological determinant analysis of the persons with this new peculiar WB pattern revealed that seroprevalence was independent from age, sex, or ethnicity, thus resembling the indeterminate profile HGIP rather than HTLV-1. Moreover, this new pattern persists over time.

Human T-cell leukemia virus type 1 (HTLV-1), simian T-cell leukemia virus type 1 (STLV-1), HTLV-2, STLV-2, STLV-3, and the recently discovered HTLV-3 and HTLV-4 constitute a group of related human and simian deltaretroviruses (37). These primate T-lymphotropic viruses (PTLVs) share common epidemiological, biological, and molecular features (37). HTLV-1 is the causative agent of adult T-cell leukemia/lymphoma (ATLL) (32, 53), a T lymphoproliferation of very bad prognosis, and of tropical spastic paraparesis/HTLV-1-associated myelopathy (TSP/HAM), a severe chronic neuromyelopathy (26). HTLV-2, which has some transforming capacities *in vitro*, has *in vivo* been associated only with rare cases of TSP/HAM-like diseases and with lymphocytosis (56).

HTLV-1 is endemic in specific geographical areas, with a total of up to 15 million to 20 million people infected. In areas where HTLV-1 is highly endemic, the seroprevalence ranges from 2 to 30% in adults and increases with age, especially in women (29, 55, 65).

Diagnostic methods used for the study of HTLV-1/2 infection include mainly serological assays searching for antibodies directed specifically against different HTLV-1 antigens (3, 25, 67). Screening tests are usually enzyme-linked immunoassorbent assays (ELISAs) (3, 5, 18, 43, 66) or particle agglutination (PA) (33). Confirmatory tests are immunofluorescence (IF) (23) but mostly Western blot (WB) analyses (30, 35, 38, 39, 64, 71). Moreover, research of integrated provirus, in the DNA from peripheral blood cells, could be done by qualitative and/or quantitative PCR (2, 4, 8, 68). Despite some improvements in the WB assays specificity during the last two decades, indeterminate serological patterns are frequent following WB analysis and represent a major concern for routine screening in blood banks in Europe, the Americas, and Africa (7, 10, 11, 16, 20, 40, 61, 63). It is also an important issue for comparative analysis between epidemiological studies performed in areas with low and high endemicity, especially in intertropical areas. The significance of these frequent indeterminate WB can be various but, in the majority of the cases, remains mostly unknown and a matter of discussion (24, 28, 57, 69). Indeed, in rare cases, these patterns have been associated with (i) HTLV-1 but mostly HTLV-2 infection exhibiting an atypical HTLV serology (6, 34, 44, 52, 68, 73, 74), (ii) HTLV-1 seroconversion (17, 45, 46), and (iii) infection by a different HTLV, such as HTLV-3 or HTLV-4 (12, 13, 42, 62, 72). Furthermore, some have been considered the results of cross-reactivity against other microbial agents, especially *Plasmodium falciparum* in Central Africa and Indonesia (31, 41, 54). Different projects on human (HTLV) and simian (STLV and foamy viruses) retroviruses have been set up in our laboratory during the last 2 decades in rural South Cameroon (9, 12, 27, 48).

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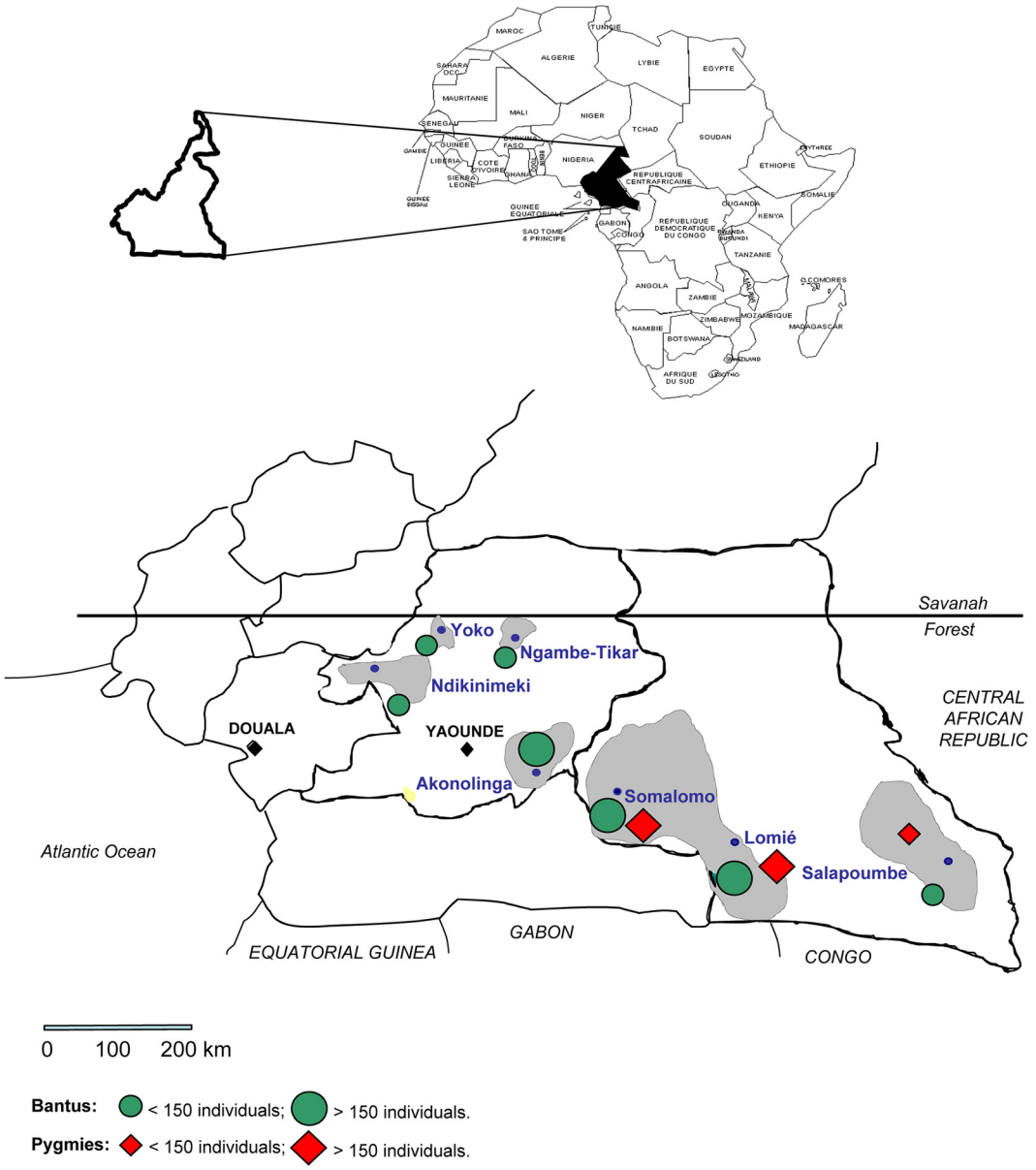

FIG 1 Map of South Cameroon and localization of the villages and settlements inhabited by Bantu and Pygmy populations included in this study.

This region (Fig. 1) is an area of endemicity for different human retroviruses, including HTLV-1 and HTLV-2, as well as the recently described new human HTLV-3 and HTLV-4 (12, 13, 62, 72). The serendipitous observation of a relatively frequent and peculiar WB pattern, among a large variety of indeterminate seroreactivities during previous studies in Central Africa, led us to perform the study reported here in order to (i) describe, in a large population of central African inhabitants, the various seroreactivity profiles by testing a systematic WB assay for HTLV-1/2 confirmation, employing the most commonly worldwide-used test; (ii) characterize, from a serological, epidemiological, and molecular point of view, this novel, frequent, peculiar WB HTLV pattern; and (iii) investigate such seroreactive pattern by comparative serology with different commonly used screening and confirmatory assays and by PCR using a panel of primers aimed to detect the different known HTLVs.

MATERIALS AND METHODS

Population study. This study was carried out in inhabitants of rural areas located mainly in South and East Cameroon (Fig. 1) in a rainforest region. A systematic approach for the enrolment of adults was carried out in the populations (Bantus and Pygmies) in all reachable villages and settlements, scattered alongside roads and tracks across the forest. A standardized questionnaire was used to collect epidemiological data, such as age, sex, location, and ethnicity. Prior to field sampling, individual consent was obtained after providing to the community detailed information and explanations of the study. Consent for underage children was obtained from their parents or a recognized guardian. A 5- to 10-ml whole-blood sample was collected in EDTA K2 vacuum tubes from all consenting individuals. Plasma and buffy coat were obtained 48 to 72 h after sampling and kept frozen at −80°C. A simple clinical examination was performed when requested by participants in the study. Treatment for common local ailments was given if available. A transfer to an appropriate medical facility was advised for severely ill individuals encountered on site.

TABLE 1 Population of Bantus and Pygmies from South Cameroon included in this study

Characteristic	Group	Value		
		Both men and women	Men	Women
Population	Total	1,968	990	978
	Bantus	1,165	599	566
	Pygmies	803	391	412
Mean age (yr)	Total	44	43.9	44.1
	Bantus	49.8	49.1	50.6
	Pygmies	35.6	35.9	35.2
Age range (yr)	Total	5–90	5–87	5–90
	Bantus	5–90	5–87	6–90
	Pygmies	5–83	8–80	5–83

This study received administrative and ethical clearance in Cameroon from the research division of the Ministry of Public Health and from the National Comity of Ethics and in France from the "Comité de Protection des Personnes" and the "Commission Nationale de l'Informatique et des Libertés."

HTLV serological analysis. All the plasma samples were analyzed by Western blot (MPD HTLV Blot 2.4, MP Biomedicals Asia Pacific Pte. Ltd., Singapore), and interpretation was done according to the manufacturer's instructions. Only clear bands were considered significant. The samples were classified as HTLV-1 positive, HTLV-2 positive, HTLV reactive, HTLV Gag indeterminate pattern (HGIP) (47), indeterminate (all the indeterminate samples being not HGIP), and negative. Samples with an uncertain result were analyzed in duplicate. Moreover, in some cases, plasma samples were also analyzed by another confirmatory test (INNOLIA HTLV I/II Score; Immunogenetics, Gent, Belgium) (59) and/or by ELISA (HTLV ELISA 4.0; MP Biomedicals Asia Pacific Pte. Ltd., Singapore), particle agglutination (Serodia; Fujirebio, Japan), and immunofluorescence assays. Determination of HTLV-1 antibody titers was performed by 2-fold dilutions, as previously described (14, 33).

Epidemiological analysis. Analysis of epidemiological determinants (age, sex, ethnicity) was done for the most frequent WB serological profiles: HTLV-1, HGIP, and a new observed indeterminate profile. Statistical analysis was performed using the software STATA (StataCorp LP, College Station, TX).

HTLV molecular analysis by PCR. DNA was extracted from peripheral blood buffy coat (PBBC) by the QIAamp blood minikit (Qiagen, Hilden, Germany). All DNA samples were tested by PCR with the human beta-globin primers. A combination of 3 PCRs using generic primers for HTLV-1/2/3/4 (tax classic, tax nested, pol nested) and 1 specific PCR for HTLV-3 (LTR nested) were performed, as previously described (12, 13, 62, 72). Nested PCR was specifically performed for the recently discovered STLV-3 Cmo8699AB and Cni7867AB (60, 75), using as a positive control a plasmid containing the target region (380 bp in the tax region) that was designed in our lab and synthesized by Eurofins MWG Operon (Ebersberg, Germany).

PCR was considered positive when amplicons from at least one amplification reaction were clearly detectable following agarose gel analysis. Amplicons from positive PCR were subjected to sequencing either directly or following cloning (TOPO cloning kit; Invitrogen, Carlsbad). The obtained sequences were then analyzed by MacVector software 9.0 (Mac Vector Inc., Cary, NC) and submitted to BLASTN.

***Plasmodium falciparum* studies.** Plasma showing a new indeterminate pattern following HTLV WB analysis were subjected to an additional study to search for a possible cause of this frequent profile, as previously described for the HGIP pattern (41). Plasma of persons exhibiting a typical HTLV-1 and HGIP WB profile were used as controls during this assay. Two immunoaffinity columns based on cyanogen bromide-activated Sepharose B (GE Healthcare, Bio-Sciences AB, Uppsala, Sweden) were prepared in parallel with antigenic proteins either from enriched *Plasmodium falciparum* schizonts (FUP/CB strain) or uninfected erythrocytes (red blood cells [RBC]) as described previously (41). All the plasma were diluted to 1:50 in 500 μl of phosphate-buffered saline (PBS) and incubated in 100 μl of each column for 30 min at room temperature on a rocking platform. The absorbed plasma was collected for analysis following centrifugation. Columns were then subjected to washing and elution (41). HTLV-1 WB assay was performed on the absorbed and eluted fractions for both columns, as described above. Moreover, for some samples, a homemade WB for *Plasmodium falciparum*, by using strips containing either schizont-infected RBC or uninfected RBC antigens, was performed in parallel on all the fractions.

RESULTS

Population studied. The studied population comprised 1,968 persons, including 1,165 Bantus (599 men and 566 women) and 803 Pygmies (391 men and 412 women), with mean ages of 49.8 and 35.6 years, respectively. Bantus originated from several groups, while Pygmies were only of the Baka tribe, the most important Pygmy group living in Cameroon (Table 1, Fig. 1 and 2).

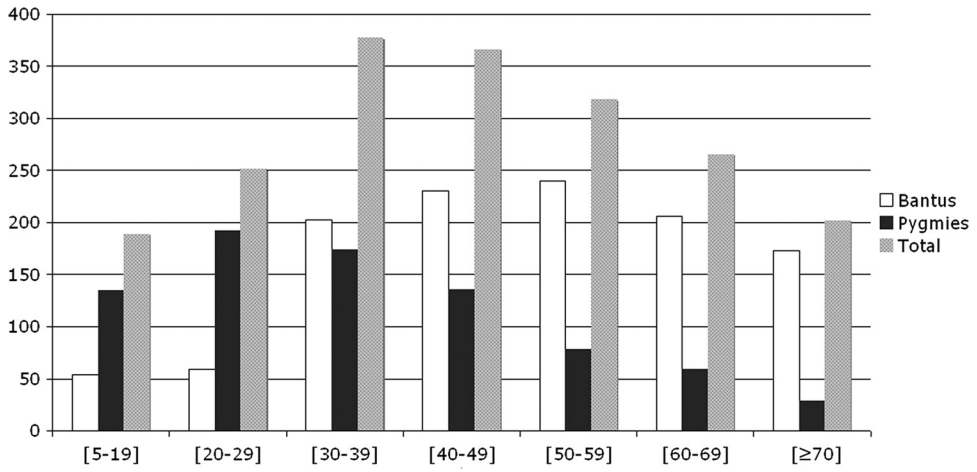

FIG 2 Age distribution of the population included in this study.

Serological analysis by Western blotting. Following Western blot analysis, 38 plasma samples out of 1,968 were considered HTLV-1 seropositive (1.93%), 13 (0.66%) were HTLV-2 seropositive, 6 (0.3%) were HTLV seroreactive, 1,292 (65.65%) were HTLV sero-indeterminate, and 619 (31.45%) were considered HTLV seronegative. Among the samples showing an HTLV indeterminate pattern, 104 plasma samples (5.28%) exhibited a typical HGIP, and 68 (3.45%) showed a frequent, yet unreported, new profile, tentatively named N. The other indeterminate samples included a combination of patterns with reactivity for at least one antigen, mainly p19 or p24 or GD21 (Fig. 3 and Table 2). The two characteristic bands of the N indeterminate profile were (i) a strong signal, localized slightly above that of the GD21 band and tentatively named shifted GD21*, and (ii) a strong p28. Signals, at different levels of intensity, for other antigens (p26, p32, p36) and reactivity to the synthetic peptides could also be present (Fig. 3).

Epidemiological determinants of HTLV-1, HGIP, N HTLV indeterminate pattern. Analysis of epidemiological determinants pointed out the classical increase of HTLV-1 seroprevalence with age ($P = 0.02$). Furthermore, HTLV-1 infection was more frequent among Pygmies (2.86%) than among Bantus (1.29%) ($P = 0.01$) (Fig. 4A and B). Although HTLV-1 seroprevalence was slightly higher in women than in men (2.04% versus 1.81%), the difference was not significant ($P = 0.71$). In contrast and, as expected, HGIP seroprevalence (Fig. 4C) did not increase with age ($P = 0.12$) and was not different according to sex ($P = 0.95$). It appeared, however, to be slightly higher among Bantus (6.18%) than among Pygmies (3.99%) ($P = 0.03$). Epidemiological analysis of the persons with the N indeterminate WB profile showed that seroprevalence was not associated with age (Fig. 4D), sex, ethnicity ($P = 0.64, 0.49, 0.12$, respectively), or geography.

PCR analysis. All the 677 tested PBBC DNAs were found amplifiable with the beta-globin primer pair. HTLV molecular analysis was then performed by PCR using different HTLV primer pairs on the PBBC DNAs of all the persons whose plasma showed a WB seropositivity to HTLV-1, HTLV-2, and HTLV. It was also performed for a series of DNAs originating from persons with an HTLV indeterminate WB, including 41 with an HGIP, 40 with the N indeterminate pattern, and 527 with other WB patterns (Table 3). Thus, among the 1,968 individuals included in this study, DNAs from 677 persons (34.4% of the entire population) were tested by PCR. The results indicate that 32 out of the 38 (84.2%) DNA samples originating from individuals with HTLV-1-positive WB and 4 out of 6 (66.7%) DNA samples originating from persons with an HTLV seroreactive profile were found HTLV-1 positive by PCR. Confirmation of the presence of HTLV-1 was obtained by sequencing of either tax or pol amplicons in all positive PCR cases. No DNA from persons whose plasma showed HTLV-2, HGIP, or indeterminate patterns was PCR positive. Additionally, no DNA was found positive for HTLV-3 or -4. Interestingly, none of the DNA samples originating from 40 persons with the N HTLV indeterminate WB pattern was positive by PCR.

Persistence over time of the N HTLV indeterminate WB profile. Fifteen persons (mean age, 39 years; 6 women/9 men) whose plasma exhibited an N indeterminate profile were sampled for a second time within the next 1 to 5 years (mean, 4 years). Moreover, a descendant (daughter or son) and the partner (wife or husband) were sampled for 8 and 2 cases, respectively. Western blotting results were comparable to the previous ones, except in two cases, in which the signal was slightly weaker. PCR assays on the PBBC DNAs of the 15 persons, by using all the five different HTLV primers pairs, were found negative, as observed in the previous DNA samples. Furthermore, the 10 plasma and DNA samples, from either the descendants or the partners of the individuals with an N profile, were all found negative following both WB and PCR.

Use of other screening and confirmatory HTLV tests for investigation of the N WB indeterminate profile. The 68 plasma samples presenting the N profile were analyzed by the two frequently used HTLV screening tests: ELISA and PA. Ten WB HTLV-1-positive and 10 WB-negative samples were also used as controls in the same experiment. All controls that were HTLV-1 positive were found seropositive with both assays, and none of the negative controls were positive. Among the 68 plasma samples with an N profile, 16.2% (11 cases) were found ELISA positive, and 10.3% (7 cases) were PA positive. Among these, 3 were positive with both ELISA and PA. However, the optical density values obtained in the ELISA were significantly lower for most of the N profile plasma samples (9 out of 11) than for the WB-positive HTLV-1 samples.

Additionally, 32 plasma samples presenting an N profile were analyzed by the other confirmatory test, INNO-LIA HTLV I/II Score. As described in Fig. 5, none of them was positive, but they were mostly negative (21 cases) or indeterminate (11 cases); the latter group included either samples with a strong reactivity for the p19 or with a variety of faint bands.

Study of cross-reactivity with *Plasmodium falciparum* in sera with an N pattern. A series of nine plasma samples exhibiting the N indeterminate pattern on Western blot analysis, as well as five HTLV-1 and seven HGIP samples, were loaded on a Sepharose column coupled with *Plasmodium falciparum* schizont-infected red blood cells. A column coupled with uninfected red blood cells was used as a control in the same experiment. The original plasma, together with the absorbed and eluted fractions, was tested by HTLV WB analysis (Table 4). All the HTLV-1 plasma samples showed a strong WB reactivity before and after incubation on the column, while the eluted fraction was mostly negative (Table 4). Almost all the plasma samples presenting the N profile showed a decrease of the HTLV WB intensity in the absorbed fraction, and in particular for samples 4 and 7 this was observed only for the schizont-absorbed plasma (Table 4), indicating a contribution of *Plasmodium falciparum* antibody cross-reactivity to the peculiar new HTLV-1 WB profile. The other plasma samples from this group had their specific HTLV WB reactivity unaltered after absorption on the *Plasmodium falciparum* column, suggesting an absence of cross-reactivity. Furthermore, most HGIP plasma samples (6 out of 7) showed a slight decrease of the WB reactivity after absorption on the schizont column, but surprisingly this was also frequently observed with the RBC-absorbed plasma, consistent with a nonspecific binding either to the column matrix or to red blood cell antigens. Moreover, in most cases, we did not observe any WB reactivity of the eluted antibodies, which reacted by Western blotting with *Plasmodium* antigens (data not shown). Thus, either the eluted anti-*P. falciparum* antibodies failed to cross-react with HTLV-1 antigens or the cross-reacting antibodies may have not been eluted from the column or were damaged during elution. All together, the data indicate that there is no link of causality of *Plasmodium falciparum* antibodies to the N indeterminate pattern.

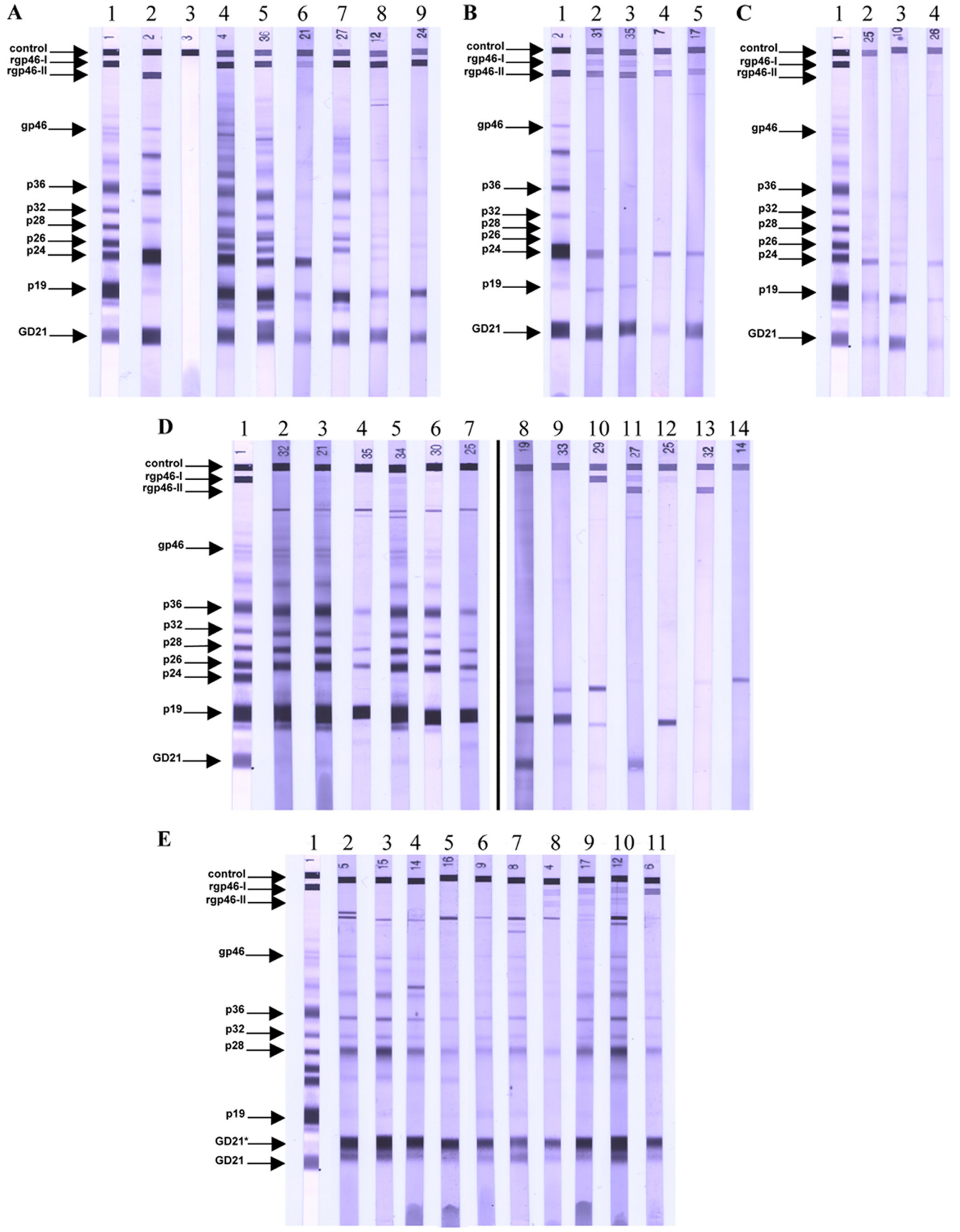

FIG 3 Representative sero-reactivity pattern using the MPD HTLV Blot 2.4 kit which contains a recombinant GD21 (common for HTLV-1 and HTLV-2) and two synthetic peptides (rpg46-I and rpg46-II), specific either for HTLV-1 or HTLV-2. (A) 1, control HTLV-1; 2, control HTLV-2; 3, negative; 4 and 5, HTLV-1 with strong reactivity; 6, HTLV-1; 7 to 9, HTLV-1 with no or faint p24; (B) 1, control HTLV-2; 2 and 3, HTLV-2; 4 and 5, HTLV-2 without p19; (C) 1, control HTLV-1; 2 to 4, HTLV; (D) 1, control HTLV-1; 2 to 6, typical HGIP; 7, HGIP with faint p24; 8 to 14, indeterminate; (E) 1, control HTLV-1; 2 to 7, N HTLV indeterminate pattern; 8 to 11, N pattern with faint peptide.

TABLE 2 HTLV Western blot analysis results of the plasma samples obtained from the 1,968 individuals participating in this study[a]

Band pattern	No. of samples	Result
GD21, p19, (p24), rgp46-I	38	HTLV-1
GD21, (p19), p24, rgp46-II	13	HTLV-2
GD21, p19, p24	6	HTLV
p19, p26, p28, p32, p36	104	Indeterminate HGIP
GD21*, p28	68	Indeterminate N pattern
One band alone or combinations ≠ from HGIP and N	1,120	Indeterminate
No band	619	Negative

[a] Interpretation was done by following the manufacturer's instructions, and only clear bands were considered positive. Parentheses indicate that reactivity is not necessary. Besides the classical described Western blot pattern (HTLV-1, HTLV-2, HTLV, HGIP, negative), we defined a new pattern, tentatively named N, among the indeterminates. According to the manufacturer's instructions, HTLV-1 and -2 and indeterminate profiles may present reactivity to p26, p28, p32, p36, and p53. According to our observations of the N pattern, reactivity to p26, p28, p32, p36, and p53 may also occur. *, reactivity localized at a shifted position slightly above the classical GD21 described from the WB manufacturer and observed in all cases of the N indeterminate pattern.

DISCUSSION

The discovery of an HTLV indeterminate serology by Western blotting always raises questions during sero-epidemiological studies and clinical medical practice (1, 6, 10, 20, 46, 52, 61). Indeed, the questions of how this pattern should be interpreted and what the practical consequences are for the person with such serological pattern remain, in most cases, a matter of debate (28, 40). Such HTLV indeterminate WB patterns have been reported, for the last 20 years, by several authors and are found mainly, and their signification studied, in tropical areas, such as Central Africa and Southeast Asia (24, 28, 31, 41, 47, 54). However, they have also been reported in other areas, such as European countries (68), the United States (11, 51, 73), the Caribbean area (17, 57), Central and South America (Brazil, Argentina) (6, 35, 44, 50, 61), and more recently the Middle East (74). Most of the studies concern blood donors (10, 11, 16, 63) or epidemiological work in the general population (43) but also organ donors for transplantation (22, 36), lactariums (19, 58, 70), and neurological patients (1, 73). Indeed, the finding of an indeterminate WB HTLV serology raises questions not easy to be resolved by the medical doctors in charge of the persons with such an indeterminate pattern.

HTLV indeterminate serologies observed using Western blotting comprise a large variety of patterns, ranging from unique and faint bands (mainly p19 or p24) to complex patterns exhibiting multiple clear bands (44, 46, 74). We have previously identified and characterized a frequent pattern that we named HGIP, for HTLV Gag indeterminate pattern (47). By performing epidemiological and molecular analyses, we have demonstrated that such reactivity was neither associated with HTLV-1 nor HTLV-2 infection (41). The description and the interpretation of such HGIP is now included in the most commonly used WB assay (MPD HTLV Blot 2.4).

The analysis of thousands of HTLV WB during several epidemiological studies performed in the last few years in different areas, especially Central Africa, led us to the serendipitous observation of a quite frequent and homogenous WB pattern, not yet reported. In this study, we tried to define and characterize such a pattern on serological, epidemiological and molecular stand-

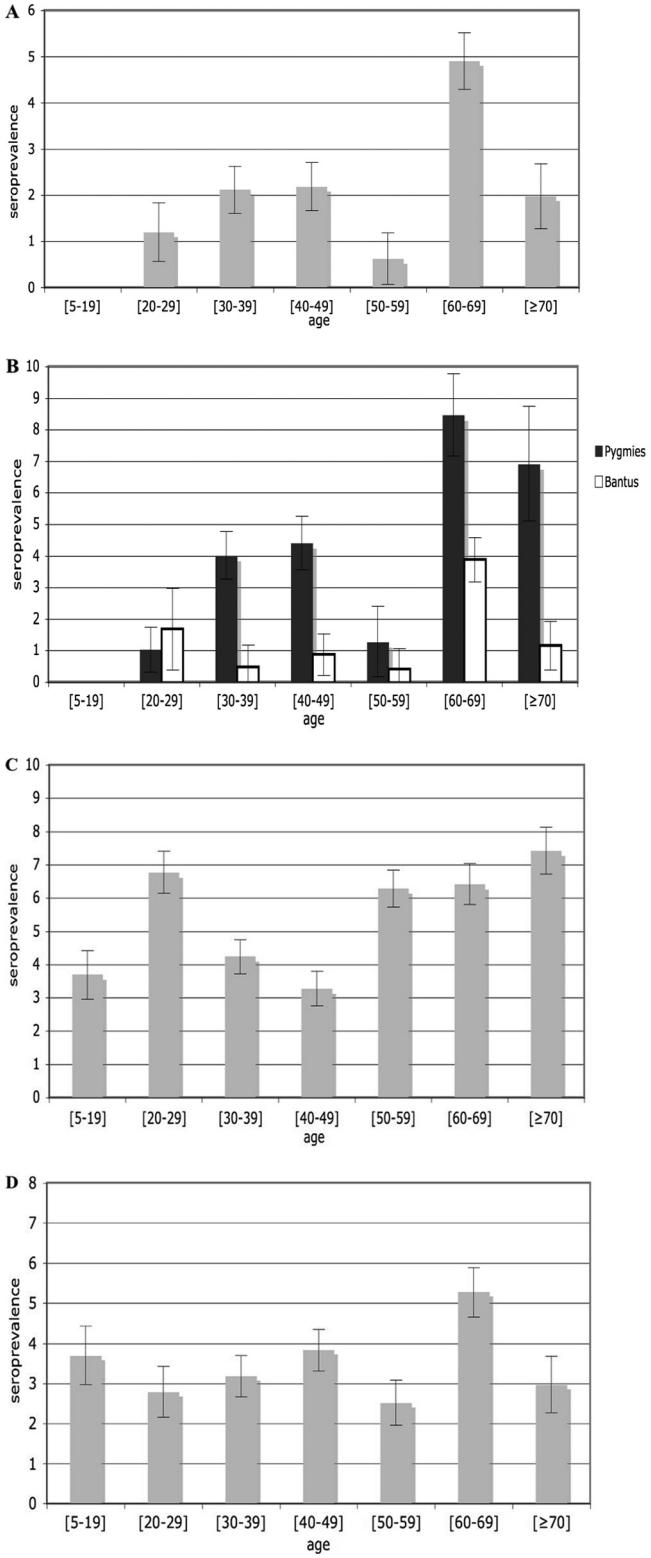

FIG 4 Seroprevalence of the different HTLV WB pattern according to age. (A) HTLV-1 in the total population; (B) HTLV-1 according to ethnic group (Pygmies and Bantus); (C) HGIP in the total population; (D) N indeterminate profile in the total population.

TABLE 3 HTLV polymerase chain reaction results versus HTLV Western blot results of 677 out of 1,968 individuals from South Cameroon included in this study

	No. of samples							
PCR result	HTLV-1	HTLV-2	HTLV	HGIP	N pattern	Other indeterminate	Negative	Total
PCR positive	32	0	4	0	0	0	0	36
PCR negative	6	13	2	41	40	527	12	641
Total	38	13	6	41	40	527	12	677

points. This was based on a comprehensive study performed on a large population living in South Cameroon. The main results of this study, in which we performed a systematic WB on all the plasma samples without any initial screening assays, are as follows.

(i) The proportion of plasma exhibiting an indeterminate WB profile is very high (65%), with a large variety of patterns, including HGIP.

(ii) The new WB pattern reported here is defined mainly by strong reactivity at a shifted position slightly above the usual GD21 (tentatively named GD21*) and against the p28. It can be associated or not with reactivity against other Gag-encoded proteins, such as p26, p32, p36, and p53, and possibly some faint reactivity against peptides MTA-1 and/or K55 (Table 2 and Fig. 3E).

(iii) This pattern, named N, is quite frequent, as it was found in nearly 4% (3.45%) of the plasma of the villagers tested. In our study, it is slightly less frequent than the HGIP (5.28%) but more frequent than the HTLV-1-seropositive pattern (1.93%).

(iv) The epidemiological investigation of persons exhibiting such WB reactivity indicates similar results to those found in persons exhibiting an HGIP but different from persons infected by HTLV-1. Indeed, it is present in all age groups and does not increase with age. Furthermore, it affects equally men and women, as is also usually found for HGIP (47). In contrast, HTLV-1 infection is generally more frequent in women, especially after 40 years of age (29, 55, 65). An important point, also observed for HGIP, is that the pattern was consistent and persistent over time. Indeed, in this study, the N indeterminate pattern persists nearly unchanged in most of the plasma from the 15 individuals tested after a mean period of 4 years. Thus, there is no evidence that it could be linked to an HTLV-1 seroconversion. This is also strengthened by the fact that during seroconversion following HTLV-1 primary infection, the occurrence of reactivity to p28 is always preceded by the occurrence of p19 and p24 reactivity (17, 45, 46). Lastly, it is also worth noting that analyses of large series of HTLV WB, performed more than 10/15 years ago in our laboratory, on a series of plasma samples mostly from Cameroon but also, to a lesser extent, from Central African Republic (RCA) and Zaire (now DRC), indicated at that time the presence of such a pattern in a significant percentage of the tested samples (A. Gessain, unpublished data). Lastly, we did not find any evidence of transmissibility of such a pattern. However, this conclusion is limited by the small series of family members tested and needs to be confirmed on a larger scale.

(v) The persons exhibiting the N pattern had no evidence of infection by an HTLV. Indeed, all the tested DNA from PBBCs of individuals presenting this new reactivity were found negative by using a battery of primer pairs, which can detect all the currently known HTLVs (12, 13, 62, 72). The PCR methodology that we used allowed the detection of HTLV-1 and HTLV-2 in several previous studies performed in different populations living in Southern Cameroon (48). It led us also to describe two HTLV-3 strains in the same area (12, 13). In the present study, the great majority (85%) of the PBBCs from individuals exhibiting an HTLV-1 serology by WB was found, as expected, to be HTLV-1

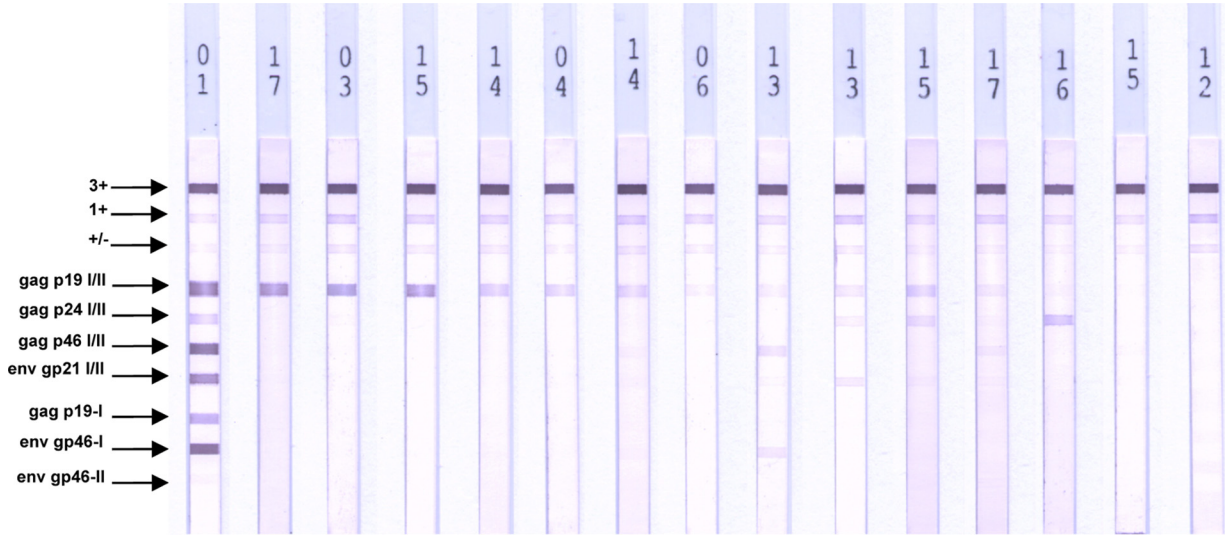

FIG 5 Sero-reactivity pattern using the INNO-LIA HTLV I/II Score (Innogenetics) performed in a group of representative samples with the N indeterminate pattern.

TABLE 4 Analysis of the cross-reactivity with *Plasmodium falciparum*, following immuno-affinity absorption into schizont-infected or uninfected erythrocyte columns

Sample	Result[a]				
	Plasma	Absorbed plasma (schizonts)	Absorbed plasma (RBC)	Eluted fraction (schizonts)	Eluted fraction (RBC)
HTLV-1					
1	+++	+++	+++	−	−
2	+++	+++	+++	−/+	+/+
3	+++	+++	+++	−	−
4	+++	+++	+++	−	−
5	+++	+++	+++	ND	ND
HGIP					
1	+++	++	++	−	−
2	+++	+	++	−	−
3	+++	++	++	+	−
4	+++	+++	+++	−	−
5	+++	++	++	−	−
6	+++	++	++	ND	ND
7	++	++	++	ND	ND
N pattern					
1	+++	++	++	+	−
2	++	−/+	−/+	−/+	−
3	+++	++	++	−	−
4	+++	+	++	−	−
5	++	+	+	−	−
6	++	++	++	ND	ND
7	+++	+	+++	ND	ND
8	+++	+	+	ND	ND
9	+++	++	++	ND	ND

[a] +++, strongly reactive; ++, reactive; +, weakly reactive; −, not reactive; −/+, different result depending on the amount of fraction used for the WB; ND, not done.

PCR positive. The few negative cases probably reflect a very low HTLV-1 proviral load, most likely below the sensitivity of our PCR assay. This is strengthened by the observation that the HTLV-1 antibody titers, as determined by IF, were lower in the 8 WB-positive (6 HTLV-1-positive; 2 HTLV-reactive) but PCR-negative samples than in the 25 WB-positive (21 HTLV-1-positive; 4 HTLV-reactive) PCR-positive samples (data not shown). Furthermore, the comparative study of the WB band intensity leads to the same conclusion.

Additionally, we performed a routinely used, seminested PCR specific for HTLV-1 (49) targeting a region of 522 bp in the gp21 *env* gene, conserved among the HTLV-1 viral strains circulating in Cameroon. No proviral HTLV-1 DNA was found in the buffy coats of individuals showing the N indeterminate WB pattern. Moreover, it is noteworthy that four out of the six DNA samples extracted from individuals with HTLV-1 serology, but negative after the previous generic PCRs, showed a signal following this specific PCR (data not shown).

Interestingly enough, 4 samples with HTLV reactivity restricted to GD21, p19, and p24 (but without specific peptide) were also detected as HTLV-1 positive by PCR. This indicates that a certain percentage of the HTLV-1-infected individuals did not present any MTA-1 peptide reactivity, as reported in a few studies (6, 15, 21, 24, 74). Furthermore, our study also indicates that, at least in Central Africa, the presence of HGIP is not associated with an infection by HTLV-3 or HTLV-4. Lastly, no sample presenting a typical HTLV-2 WB profile was found positive by PCR. This confirms previous observations concerning the absence of this virus among Bantus and Baka Pygmies inhabiting this area (48). The significance of the observed HTLV-2-like WB pattern, however, remains to be elucidated.

In this study, in order to detect all indeterminate serologies, we tested systematically by WB analysis the plasma samples of the 1,968 persons participating in this epidemiological work. However, WB is used mainly as a confirmatory assay and performed only on samples first found positive by screening tests (mostly by ELISAs but also by PA). The two tests differ in the antigens used to detect the presence of antibodies: a viral lysate in the case of the PA, and recombinant and synthetic proteins for the ELISA. Thus, an important question was whether the samples exhibiting the new WB pattern were positive for the HTLV screening assays most commonly used in blood banks and in medical practice. Interestingly, in this series, 7 (11%) and 11 cases (16%) of the 68 plasma samples exhibiting the N HTLV indeterminate pattern were found positive by PA and ELISA, respectively. Among them, 3 were positive by both assays. This indicates that a certain proportion of such blood samples will be detected by screening assays and will be tested by a confirmatory assay, such as WB analysis or INNO-LIA.

To find out the possible causes of the new WB pattern reported here, research on the association and possible cross-reactivity with *Plasmodium falciparum* antigens was done by immuno-affinity chromatography assay, as previously shown for the HGIP profile (41), with different controls. Although a decrease of the WB reactivity of the schizont-absorbed plasma was observed for some N indeterminate profile samples reported here (Table 4), this was not consistently observed with all plasma. Our data do not argue for a clear causal association of such a pattern with the presence of antibodies against *Plasmodium falciparum*. Additional cross-reactivity studies are thus necessary in order to decipher the origin of the frequent HTLV indeterminate serology, including the new pattern described in this study.

In conclusion, a systematic HTLV Western blot screening of a large population living in rural South Cameroon allowed the confirmation of the epidemiological characteristics of the persons exhibiting an HTLV-1 and HGIP WB profile. Most importantly, we describe for the first time a frequent new indeterminate HTLV WB pattern, tentatively named N, which was found in some individuals scoring positive in one of the other serological assays performed during screening. The fact that the epidemiological determinants of the persons showing such patterns are different from those infected by HTLV-1 and the negative PCR results strongly suggest that this new HTLV reactivity is not linked to an infection by a retrovirus related to an HTLV-1.

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